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Transdermal delivery of paracetamol for paediatric use: effects of vehicle formulations on the percutaneous penetration

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Abstract

Paracetamol is a safe and effective analgesic and antipyretic agent, and is one of the most widely used medications for infants and children. The formulations currently available have been designed for oral and rectal administration. However, they are not practical in young patients with vomiting and diarrhoea, or in those who refuse to take the full dose. An alternative route of administration would be a significant contribution to the paediatric pharmacopoeia. The aim of this study was to develop a new transdermal system for optional therapeutic administration of paracetamol in infants and children. In-vivo studies were carried out in animals using a transdermal system of high-loaded, soluble paracetamol in a hydrogel patch, which was also tested in-vitro for 8 h. Although the beneficial contribution of glyceryl oleate to the transdermal penetration of paracetamol seemed to be significant in-vitro, it was shown to be insufficient in-vivo. To improve the penetration of the drug, 4% PEG-40 stearate and 10% ethanol were incorporated as absorption enhancers into the dermal patches. A few hours after application of the improved patches to rats, plasma drug concentrations were elevated to levels comparable with those obtained after oral and subcutaneous administration of a high dose of paracetamol. Since plasma drug concentrations did not reach a constant steady state (as a peak or plateau) during the short-term animal experiments, longer pharmacokinetic studies in conscious animals are necessary.

Introduction

Paracetamol (N-acetyl-p-aminophenol) is one of the most widely used medications for infants and children, and its antipyretic and analgesic efficacy is well established in all age groups. After oral administration of therapeutic doses, paracetamol is eliminated mainly as glucuronide and sulfate conjugates in the ratio of about 2:1. A small proportion (about 7% of the dose) undergoes oxidation by microsomal mixed-function oxidases to a highly reactive intermediate N-acetyl-p-benzoquinoneimine (Miner & Kissinger 1979), which is detoxified by reaction with sulfhydryl groups, such as reduced glutathione, and excreted in the form of mercapturate and cysteine conjugates. After overdosage, hepatic glutathione is depleted and N-acetyl-p-benzoquinoneimine reacts with sulfhydryl groups of hepatic proteins leading to hepatic necrosis (Insel 1996).

Apart from the potential toxicity, the existing oral or rectal routes of administration of paracetamol may not be practical in infants and children with gastroenteritis because of vomiting and even diarrhoea in acute cases. Oral administration may also not be practical in young patients who often refuse to take the full dose of the medication orally (because of taste or other reasons). Since paracetamol is poorly water soluble, no intravenous or intramuscular paracetamol formulations are available, although the intravenous application to adults is marketed in some European countries as a soluble prodrug named propacetamol. Thus, an alternative route of administration would be most useful for paediatric use. In general, transdermal administration of medication provides several potential advantages, including elimination of variations in plasma concentrations after gastrointestinal absorption, elimination of the hepatic first-pass metabolism, a decrease in side-effects, and avoidance of a

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gastrointestinal intolerance (Chien 1987; Berner & John 1994). In addition, this route of administration is able to maintain prolonged blood concentrations at therapeutic levels and it enables drug input termination in cases of intoxication. This route of administration may be of particular significance in infants and children because of their greater surface area to weight ratio. The epidermis of the full-term neonate (but not that of the premature infant) is well developed and similar to that of an older child or adult. However, the thinner skin of the infant and child, with its relatively rich blood supply, may affect the pharmacokinetics of drugs administered by transdermal delivery systems; this has obvious therapeutic advantages, but it may also have toxicity implications (the majority of cases of percutaneous drug toxicity have occurred in infants: aniline dye, hexachlorophene, iodine and alcohol poisoning). To the best of our knowledge, no published information exists on transdermal administration of paracetamol in infants and children.

We present for the first time an attempt to deliver paracetamol in its soluble form by using high-drug loaded transdermal formulations. In this preclinical study, we investigated mainly the in-vivo percutaneous absorption and, to a limited extent, the in-vitro percutaneous penetration of paracetamol through animal skin. We report on how several commonly used absorption enhancers affect the skin permeability to paracetamol. Accelerants such as glyceryl oleate, ethyl alcohol, PEG-40 stearate and PEG-40 hydrogenated castor oil were examined for their contribution to drug delivery from transdermal systems.

Materials and Methods

Materials

Paracetamol was donated by Vitamed Pharmaceuticals Ltd, Israel. PEG-40 stearate and glyceryl oleate were obtained from Uniqema, Bromborough Pool, The Wirral, UK. Isopropyl myristate and tetraglycol were purchased from Sigma, Rehovot, Israel. Guar-based polymer (guar gum, 2-hydroxypropyl 2-hydroxy-3-(trimethylammonio)propyl ether chloride; CTFA/INCI name: hydroxypropyl guar hydroxypropyltrimonium chloride) was purchased from Rhone-Poulenc, Paris La Defence, France. Polyoxyl 40 hydrogenated castor oil USP24/NF19 (PEG-40 hydrogenated castor oil, Chremophor RH40) was obtained from BASF, Ludwigshafen, Germany. High-performance liquid chromatography (HPLC) grade solvents were obtained from Merck (Darmstadt, Germany). Ketamine hydrochloride injections USP (equivalent to 100 mg mL^{-1} ketamine) were used from Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA.

Preparation of transdermal patches containing paracetamol

Round paracetamol patches containing 100 mg drug per 1.8 cm^2 surface area (15 mm diam., 3 mm thick) were prepared by moulding paracetamol solutions containing guar-based polymer for 1 h at ambient temperature (Sintov & Gorodischer 2000). The solutions were prepared by dissolving paracetamol in combinations of tetraglycol and glyceryl oleate (2.2:1) or tetraglycol, glyceryl oleate and isopropyl myristate (3.4:1:1). Just before moulding, the polymer (50–60 mg per patch), distilled water, PEG-40 stearate solution, and ethyl alcohol were mixed into the paracetamol solution. The mixture was then poured into the moulds while checking the uniformity using a semi-analytical balance. An in-vitro release testing of the formed patches at sink conditions demonstrated immediate dissolution of paracetamol into the aqueous medium (data not shown).

In-vitro skin permeation study

The permeability of dermal paracetamol through hairless mouse skin was measured in-vitro with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ, USA). The diffusion area was 1.767 cm^2 (15 mm diam. orifice), and the receptor compartment volumes varied from 11 to 12 mL. The solutions on the receiver side were stirred by externally driven, Teflon-coated magnetic bars. Each set of experiments was performed with six diffusion cells. Sections of full-thickness hairless mouse (CD1 strain, male, 6–7 weeks old; Weizmann Institute, Rehovot, Israel) abdominal skin were excised from the fresh carcasses of animals killed with ethyl ether. Subcutaneous fat was removed with a scalpel, and the skin sections were mounted in the diffusion cells. The skin was placed on the receiver chambers with the stratum corneum facing upwards, and then the donor chambers were clamped in place. The excess skin was trimmed off, and the receiver chamber, defined as the side facing the dermis, was filled with phosphate-buffered saline (PBS; pH 7.4). After 30 min of skin washing at 37 °C, the buffer was removed from the cells. Paracetamol patches or alcoholic solutions were applied on the skin, and the receiver chambers were filled with phosphate buffer (4 mm, pH 7.4)/ ethyl alcohol (analytical grade) (1:1). Samples (2 mL) were withdrawn from the receiver solution at predetermined time intervals, and the cells were replenished to their marked volumes with fresh buffer solution. Addition of solution to the receiver compartment was performed with great care to avoid trapping air beneath the dermis. Samples were taken into 1.5-mL amber vials every hour over an 8 h period. The samples were kept at −20 °C until analysed by HPLC.

Pharmacokinetic studies of transdermal paracetamol in rats

The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee, which complies with the Israeli Law of Human Care and Use of Laboratory Animals, 1994 (also approved by the Division of Compliance, OPRR, OD of the US-NIH as a foreign institution with compliance to the standards for

Human Care and Use of Laboratory Animals, approval: A5060-01); the institution has adopted policies regarding animal care and use as outlined in the guide for the Care and Use of Laboratory Animals of the National Academy of Sciences, USA.

Male Sprague-Dawley rats (400–500 g; Harlan Laboratories Ltd, Jerusalem, Israel) were anaesthetized (100 mg kg^{-1} ketamine, i.p.) and placed on their backs. The hair on the abdomen was trimmed and the abdominal skin was gently washed with distilled water. Anaesthesia was maintained with 0.1 mL ketamine (100 mg mL^{-1}) throughout the experiment. Paracetamol patches (1.8 cm^2) were applied on the skin surface, covered and attached to the skin by an adhesive tape. In cases where skin was pretreated before application, gauze pads soaked with 0.5 mL ethyl or isopropyl alcohol were placed on the centre of the abdomen for 60 min. After drug application, blood samples (0.5–0.7 mL) were taken from the tail vein into heparinized tubes. After separation, plasma samples were kept at −20 °C until analysis. Just before analysis, 100 μL plasma was mixed with 200 μL of perchloric acid solution (6% v/v), vortexed and centrifuged at 8200 g for 5 min. The supernatant was transferred to a clean vial and injected onto the HPLC system as described below. After 8 h, the rats were killed by aspiration of ethyl ether. The drug-exposed skin areas were swabbed 3–4 times with three layers of gauze pads, washed for 30 s with running water, wiped carefully and harvested from the animals.

Skin extraction

After 8 h of the in-vivo or in-vitro studies, the drug-exposed skin sections were rinsed with phosphate buffer (pH 7.4), cut into small pieces and inserted into 2-mL vials. The skin pieces in each vial were extracted by 1 mL ethyl alcohol. Each extraction was performed by incubation in a 50 °C shaking water bath (150 rev min^{-1}) for 1 h. This extraction procedure yielded high drug recovery (> 90%). The extracts were injected onto the HPLC system.

Incubation of paracetamol with rat skin slices: in-vitro metabolism by skin

Male Sprague-Dawley rats (200–300 g; Harlan Laboratories Ltd) were killed by aspiration of ethyl ether. The abdominal hair was clipped off, the skin was excised, washed carefully with PBS (pH 7.4), and weighed. Skin tissue (3 g) was cut into small pieces (0.5–1.0 mm) with a scalpel, inserted into vials containing 2.5 mL Dulbecco's modified Eagle's medium (with 4.5 g L^{-1} D-glucose and without L-glutamine; Biological Industries Ltd, Kibbutz Beit-Haemek, Israel) and 2.5 mL paracetamol solution (50 mg L^{-1} PBS). The reaction mixture was incubated at 37 °C in a shaking bath. Samples (400 μL) were withdrawn during the incubation period into polypropylene tubes, which were immediately dipped in boiled water for 30 s. The samples were then centrifuged and the supernatants were separated and kept frozen until analysis.

HPLC analysis of samples from receiver solutions, plasma and skin extracts

For receiver solutions, samples of 20 μL from each vial were injected onto the HPLC system, equipped with a prepacked C18 column (Lichrosphere 100 CN, 5 μm, 250 × 4 mm; Merck). The HPLC system consisted of a Perkin Elmer Model LC 250 pump and diode array detector Model 235C. The quantitation of paracetamol was performed by integration of peaks detected at 245 nm. The samples were chromatographed using an isocratic mobile phase comprising water and methanol (3:1) at a flow rate of 0.5 mL min^{-1}. A calibration curve (peak area vs drug concentration) was constructed by running standard paracetamol solutions in ethanol/water for every series of chromatographed samples. Calibration curves were linear over the range 0.5–200 μg mL^{-1}. As a result of the sampling of large volumes from the receiver solution (and the replacement of these amounts with equal volumes of buffer), the receiver solution was constantly being diluted. Taking this process into account, the cumulative drug permeation (Q_t) was calculated from the following equation:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i is the drug concentration of the i^{th} sample, and V_r and V_s are the volumes of the receiver solution and the sample, respectively. Data were expressed as the cumulative paracetamol permeation per unit of skin surface area, Q_t/S ($S = 1.767$ cm^2). The steady-state fluxes (J_{ss}) were calculated by linear regression interpolation of the experimental data:

$$J_{ss} = \Delta Q_t/(\Delta TS)$$

For plasma and skin extracts, samples of 20 μL were injected onto the HPLC system, equipped with a prepacked C18 column (Betasil C18, 5 μm, 250 × 4.6 mm; Thermo Hypersil, UK). The HPLC system consisted of a Perkin Elmer Model LC 250 pump and diode array detector Model 235C. This analytical method was intentionally modified from the method used for receiver solutions to facilitate the quantification of paracetamol and its sulfated metabolite simultaneously (Brunner & Bai 1999; Campanero et al 1999). Paracetamol and its sulfated metabolite (identified by its spectrum) were eluted using an isocratic mobile phase comprising acetonitrile and potassium phosphate buffer (1 mM, pH 3.0) (10:90) at a flow rate of 1 mL min^{-1}. Calibration curves were linear over the range 0.5–20 μg mL^{-1}.

Statistical analysis

The statistical difference between the skin penetration profiles of the formulations was analysed. The two-way unweighted means analysis of variance test for the differences among group means was first run. However, this test

required a normal distribution of data. If normality of data analysed by the analysis of variance procedure failed while using the Kolmogorov–Smirnov test, the non-parametric Mann–Whitney rank sum test for unpaired data was applied at a significant level of 0.05.

Results

Formulation

The method of manufacturing the transdermal patches of paracetamol was unique by virtue of the guar-based polymer used, which solidifies the drug-containing liquid within minutes to form a patch of any size, shape and thickness. The process could be designed in such a way that once the polymer is dispersed in a liquid containing the drug, the mixture is moulded into a patch, a process that takes from a few minutes to up to an hour for some formulation types. The obtained patches were self-adhesive and adhered to the skin surface, requiring only a covering sheet with adhesive margins to protect the systems from evaporation or contamination during treatment. Paracetamol is water-insoluble and therefore its dissolution at high concentrations usually needs the incorporation of alcohol (e.g. ethanol). However, the use of solvents such as ethanol in topical preparations may lead to precipitation of the drug on the skin upon rapid evaporation and/or intradermal permeation of ethanol once applied to the skin. We have circumvented this problem first by using a combination of tetraglycol and glyceryl oleate (2.2:1) and, in a later development, by using a combination of tetraglycol, glyceryl oleate and isopropyl myristate (3.4:1:1). These combinations enabled the preparation of high loaded patches with paracetamol in its soluble form, that is 100 mg soluble paracetamol in a 0.54 cm^3 patch volume.

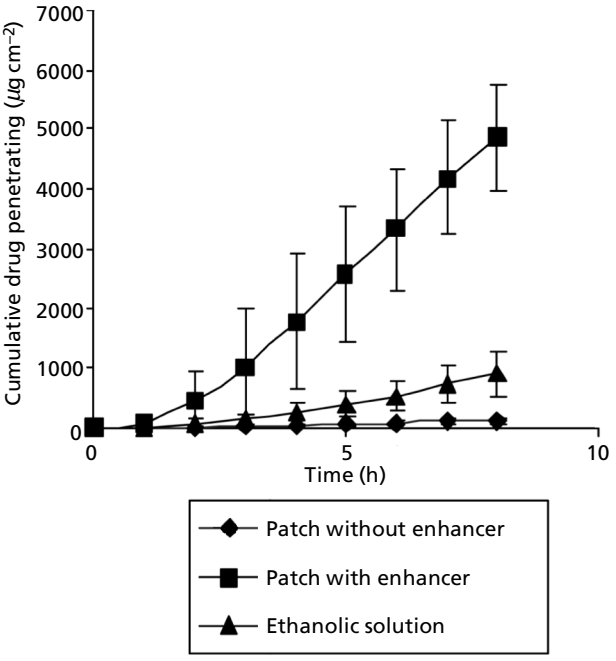

Figure 1 Percutaneous penetration of paracetamol from transdermal patches with and without glyceryl oleate as well as from an ethanolic solution of the drug after application on hairless mouse skin in diffusion cells (n = 4).

In-vitro studies

Figure 1 presents the in-vitro penetration of paracetamol through hairless mouse skin from three delivery systems: (i) a patch containing 100 mg paracetamol without penetration enhancers; (ii) a patch containing 100 mg paracetamol with glyceryl oleate as an enhancer; and (iii) an ethanolic solution (0.5 mL) containing 125 mg of the drug for the purpose of comparison. When a penetration enhancer (glyceryl oleate) was added to the formulation, the extent of paracetamol penetration was increased by approximately 25-fold ($P < 0.05$). The concentration of the drug in the donor was 188 mg cm^{-3}; therefore, the apparent permeability coefficient (J_{ss} divided by the donor concentration) rose from 9.10×10^{-5} cm h^{-1} (without the enhancer) to 4.11×10^{-3} cm h^{-1} (with the enhancer). This dramatic elevation of paracetamol permeability from the patches could not be compared with the penetration observed after dermal application of pure alcoholic solutions containing 125 mg of paracetamol (apparent permeability coefficient = 6.28×10^{-4} cm h^{-1}). It was found that 85.9 ± 37.9 μg (mean ± s.e.) of intact paracetamol was accumulated in the skin exposed to patches containing no enhancer. In comparison, the skin exposed to patches containing enhancer accumulated 1176.5 ± 323.8 μg of intact paracetamol. The extracts of the solution-exposed skin contained only half the amount of intact drug found in the skin samples treated with patches containing enhancer (501.8 ± 109.5 μg).

In-vivo studies

Although the beneficial contribution of glyceryl oleate to the transdermal penetration of paracetamol seemed to be significant in-vitro, it was found to be insufficient in-vivo, as it resulted in relatively low plasma concentrations of paracetamol (< 1 μg mL^{-1}) (data not shown). To improve the penetration of the drug, 4% PEG-40 stearate was incorporated into each patch as an enhancer. Figure 2A and B, and Table 1 show that PEG-40 stearate increased the systemic absorption of paracetamol only from patches in which 10% ethyl alcohol was incorporated into the formulation. The area under the plasma concentration–time curve ($AUC_{0,t=8\,h}$) was averaged as 25.01 ± 8.75 μg h mL^{-1} vs 5.60 ± 1.97 μg h mL^{-1} in the presence and absence of ethanol, respectively. To substantiate this result, another experiment was performed with ethanol-containing patches. As shown in Figure 2C and D, and Table 1, only those ethanolic patches that contained PEG-40 stearate resulted in a significant elevation of percutaneous paracetamol absorption into the blood.

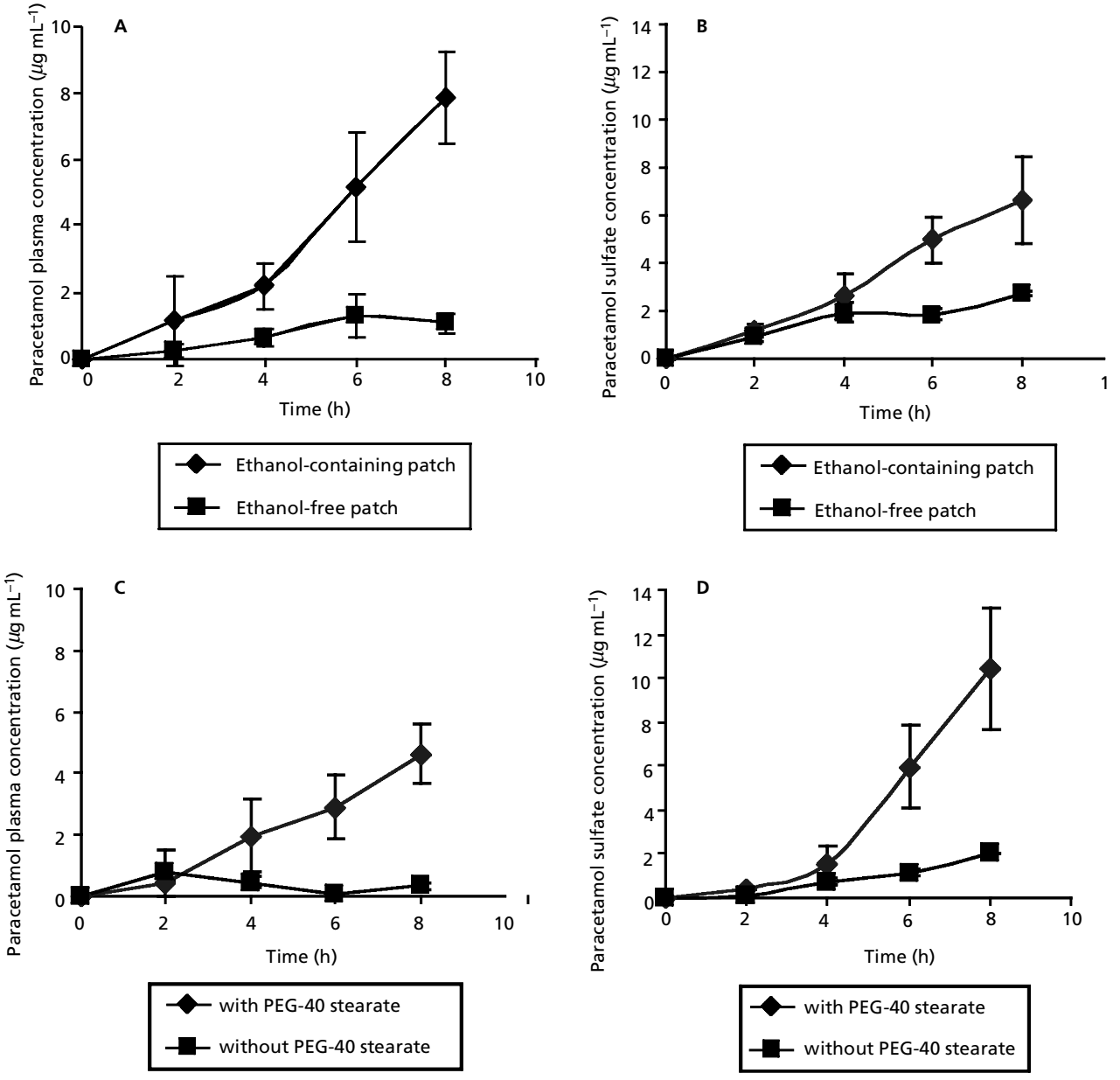

Figure 2 Plasma concentrations of paracetamol and its sulfated metabolite in rats after application of dermal patches containing 100 mg of the drug. Patches were loaded with glyceryl oleate, isopropyl myristate and PEG-40 stearate (A, B) or with glyceryl oleate, isopropyl myristate and ethanol (C, D).

Figure 3 presents another experiment attempting to compare the effect of PEG-40 stearate with the effect of another commonly used non-ionic surfactant, PEG-40 hydrogenated castor oil; both were in combination with ethanol within the patch formulation. The figure shows that PEG-40 stearate resulted in greater percutaneous absorption than PEG-40 hydrogenated castor oil. The mean $AUC_{0,t=8\,h}$ as calculated after application of a formulation containing both ethanol and PEG-40 stearate was similar to those obtained previously ($AUC_{0,t=8\,h} = 20.95 \pm 9.55\ \mu g\,h\,mL^{-1}$, $C_{t=8\,h} = 6.80 \pm 2.12\ \mu g\,mL^{-1}$) (Table 1). In comparison, the formulation that contained ethanol and PEG-40 hydrogenated castor oil was 2-fold less effective for paracetamol permeability than the formulation containing PEG-40 stearate ($AUC_{0,t=8\,h} = 10.05 \pm 2.09\ \mu g\,h\,mL^{-1}$, $C_{t=8\,h} = 3.15 \pm 0.55\ \mu g\,mL^{-1}$) (Table 1). The effect of pretreatment of the skin with alcohol before application of paracetamol patches containing the PEG-40 stearate and ethanol combination was evaluated. Pretreatment with isopropyl alcohol or ethyl alcohol before the patch application increased the penetration of paracetamol through the skin into the blood circulation

Table 1 Summary of the area under the plasma concentration–time profiles ($AUC_{0,t=8h}$) and mean plasma concentrations ($C_{t=8h}$) obtained 8 h after application of patches containing 100 mg paracetamol on rat skin.

Patch content[a]	Paracetamol		Paracetamol sulfate	
	$AUC_{0,t=8h}$ ($\mu g\,h\,mL^{-1}$)	$C_{t=8h}$ ($\mu g\,mL^{-1}$)	$AUC_{0,t=8h}$ ($\mu g\,h\,mL^{-1}$)	$C_{t=8h}$ ($\mu g\,mL^{-1}$)
PEG-40 stearate, ethanol[b]	25.01 ± 8.75	7.86 ± 1.38	24.29 ± 5.93	6.66 ± 1.80
PEG-40 stearate, no ethanol[b]	5.60 ± 1.97	1.09 ± 0.28	12.04 ± 0.77	2.70 ± 0.09
PEG-40 stearate, ethanol[c]	15.32 ± 5.64	4.63 ± 0.99	26.17 ± 8.31	10.42 ± 2.76
No PEG-40 stearate, ethanol[c]	2.92 ± 1.05	0.32 ± 0.12	6.00 ± 0.58	2.04 ± 0.02
PEG-40 stearate, ethanol[d]	20.95 ± 9.55	6.80 ± 2.12	56.47 ± 26.49	17.81 ± 6.38
PEG-40 castor oil, ethanol[d]	10.05 ± 2.09	3.15 ± 0.55	31.81 ± 6.60	9.14 ± 1.82
PEG-40 stearate, ethanol[e]	20.95 ± 9.55	6.80 ± 2.12	—	—
PEG-40 stearate, ethanol and ethanol pretreatment[e]	38.60 ± 0.69	9.65 ± 1.65	—	—
PEG-40 stearate, ethanol and isopropyl alcohol pretreatment[e]	40.38 ± 9.90	11.79 ± 4.12	—	—

[a]All patches also contained glyceryl oleate, isopropyl myristate, tetraglycol, distilled water and gelling agent; [b]see Figure 2A and B; [c]see Figure 2C and D; [d]see Figure 3; [e]see Figure 4.

(Figure 4). The skin drug accumulation after 6 h from the time of patch application increased by 2-fold with alcohol compared with the alcohol-free procedure (78.3 ± 20.3 vs 40.2 ± 11.7 $\mu g\,cm^{-2}$ skin surface area, $P < 0.05$). Following these results, we attempted to study the possible contribution of ethanol in the inhibition of paracetamol metabolism in the skin. The kinetics of paracetamol degradation by skin slices was measured with and without elevated concentrations of ethanol. As shown in Figure 5, paracetamol was metabolized in skin in-vitro, however, no change in the rate of drug disappearance was noted with ethanol.

For comparison, the plasma concentrations of paracetamol and its metabolite monitored after oral and subcutaneous administration of the drug to rats at a dose of 22 mg kg^{-1} (or 10 mg per animal) are presented in Figure 6 (see below).

Discussion

The effectiveness of any transdermal system depends on its ability to deliver sufficient amounts of drug across the skin into the blood circulation to achieve its therapeutic goal. The present study demonstrated the percutaneous delivery of significant quantities of paracetamol into the blood using a patch formulation, which contained non-irritant and pharmaceutically acceptable inactive ingredients

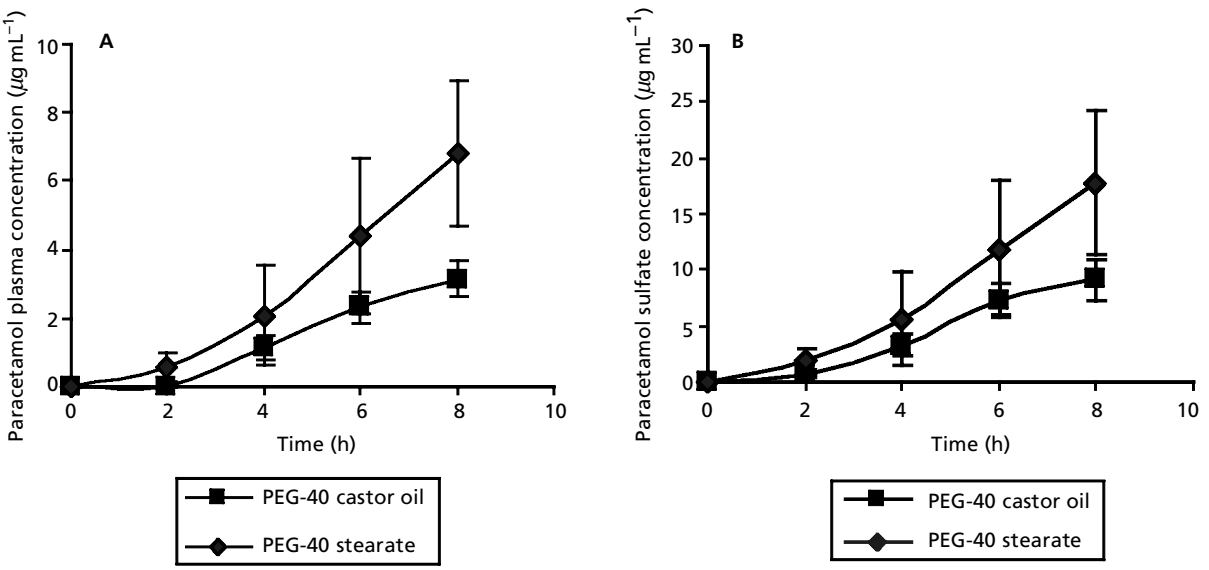

Figure 3 Plasma concentrations of paracetamol (A) and its sulfated metabolite (B) in rats after application of dermal patches containing 100 mg of the drug. The patches were loaded with glyceryl oleate, isopropyl myristate and ethanol.

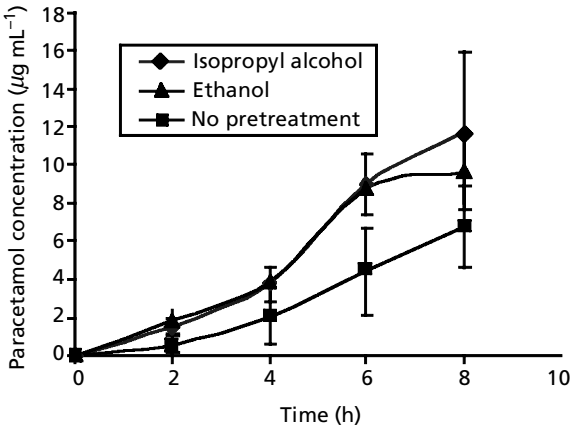

Figure 4 Plasma concentrations of paracetamol in rats after application of dermal patches containing 100 mg of drug. The patches were loaded with glyceryl oleate, isopropyl myristate, ethanol and PEG-40 stearate. The application site was pretreated with isopropyl alcohol, ethanol, or received no pretreatment.

(i.e. ethanol, isopropyl myristate, glyceryl oleate and PEG-40 stearate). After a few hours from the time of the patch application (Figures 2–4, for patches containing alcohol and PEG-40 stearate), rat plasma drug concentrations reached levels that were comparable with those obtained after oral and subcutaneous administrations of a high dose of paracetamol (22 mg kg^{-1}, 10 mg per animal) (Figure 6). Although a high drug dose was administered, the drug levels of paracetamol in the rat plasma were

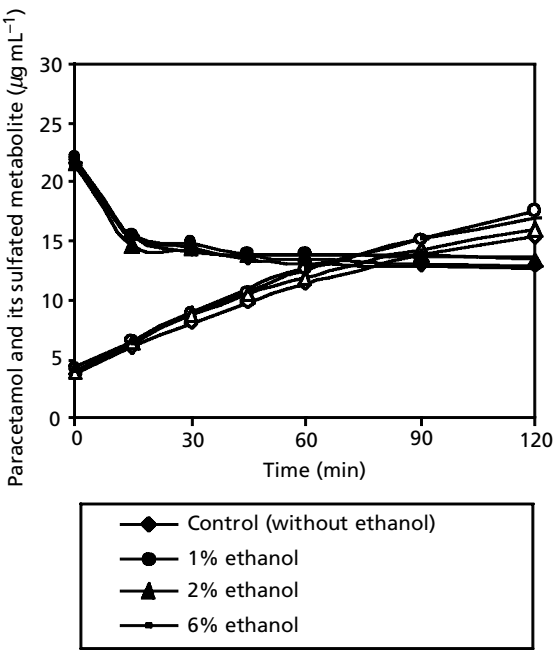

Figure 5 Degradation of paracetamol (closed symbols) and concomitant formation of its sulfated metabolite (open symbols) during incubation with rat skin slices. The effect of various concentrations of ethanol was recorded.

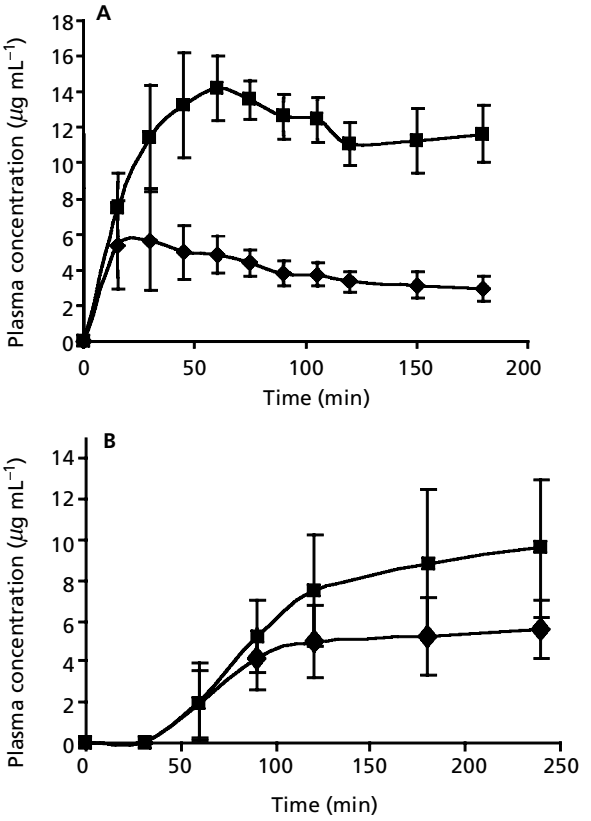

Figure 6 Plasma concentrations of paracetamol (♦) and its sulfated metabolite (■) after oral (A) and subcutaneous (B) administration of 10 mg paracetamol to rats (n = 3).

relatively low (about 6 μg mL^{-1}), presumably owing to a high rate of metabolic activity in this species (see data of the formed sulfated metabolite with each figure). The therapeutically effective levels of paracetamol are 10–20 μg mL^{-1} in human plasma. These levels are usually obtained following oral doses of 325–1000 mg and up to 4000 mg daily in adults. In infants and children, the oral administration of paracetamol includes 10–15 mg kg^{-1} every 4–6 h up to 5 times per day for not more than 10 days, or 1.5 g m^{-2} body surface area per day, in divided doses (Schachtel 1992; Insel 1996). Although the therapeutically effective levels obtained in humans were not observed in rat plasma, we have shown that rat plasma concentrations of paracetamol were quite comparable at 8 h after transdermal application of 55 mg cm^{-2} (a reservoir of 100 mg soluble drug per patch, 1.8 cm^2) and after oral or subcutaneous administration of doses of 10 mg paracetamol. This may indicate that through well-designed administration of transdermal paracetamol in humans, the desired effective levels could be achieved, particularly in children. The epidermis of the full-term neonate (but not that of the premature infant) is well developed and similar to that of an older child or adult (Harpin & Rutter 1983). However, the thinner skin and relatively rich blood supply of the infant and child can facilitate the percutaneous absorption of drugs and affect

their pharmacokinetics (Choonara 1994; Evans & Rutter 1989). Also, transdermal administration is of particular significance in infants and in children because of their greater surface area to weight ratio.

Although drug plasma concentrations were observed as being at therapeutic levels after 8 h, the in-vivo transdermal data obtained after these paracetamol patches showed that plasma concentrations had not yet reached steady state, that is the drug was still penetrating, with a concomitant elevation of systemic paracetamol. In addition to longer studies in animals (until steady state concentrations are attained), more studies should be done with patches having modified formulations and/or containing chemical enhancers to increase rates of drug penetration. However, the relatively slow increase rate of drug concentrations in the plasma might be the result of the high rate of metabolic activity (e.g. sulfation) in the skin (Figure 5). This could also be supported by the fact that paracetamol reached steady state penetration profiles after 1–2 h when it was tested on excised skin in-vitro. In these in-vitro studies, no detectable sulfated metabolite was found. Although a different animal skin was used in-vitro (hairless mouse skin), it may be that sulfation and other metabolic pathways in the skin are factors that hinder the attainment of steady state paracetamol plasma levels for at least 8 h. It should be noted that sulfation of drugs in skin tissues has already been reported. Sulfotransferase activity in organ-cultured vibrissa follicles metabolized minoxidil to minoxidil sulfate, the active metabolite that stimulates hair follicles (Buhl et al 1990; Baker et al 1994; Anderson et al 1998). Obviously, paracetamol was found to block follicular stimulation by minoxidil (Buhl et al 1990). Other reports have suggested that skin cholesterol sulfation was involved in epidermal differentiation (Epstein et al 1988; Kuroki et al 2000), and the metabolism was mediated by hydroxysteroid sulfotransferase St2b2 (Shimada et al 2002). Skin sulfation is apparently the first-pass metabolism of paracetamol administered by the transdermal route. However, apart from resulting in a delayed period to reach therapeutic levels (lag-time), this first-pass effect is minor and does not significantly affect the overall metabolism, as shown when comparing Figures 2 and 3 (transdermal administration) with Figure 6 (oral and subcutaneous administration).

Alcohol has been reported to enhance and facilitate the transport of a significant number of drugs across the skin (DeNoble et al 1987; Ghanem et al 1987; Kai et al 1990; Hatanaka et al 1993; Berner & Liu 1995; Alberti et al 2001; Oh et al 2002). Since the stratum corneum is the major diffusional barrier for transdermal transport, it has been suggested that alcohol increases the permeation of polar drugs by extracting stratum corneum lipids (Walters 1989). As shown in the present study, alcohol alone is insufficient to increase the penetration of paracetamol, perhaps because of its relatively low polarity. Therefore, surfactants such as polyethoxylated derivatives of stearic acid or hydrogenated castor oil are required to enhance the transport of paracetamol across the fluidized lipoidal pathway of the stratum corneum. Several reports suggest that ethanol can increase the percutaneous absorption of permeants by inhibition of their enzymatic transformation in the viable epidermis; thus there are more molecules of intact compounds for topical or systemic availability (Weibel & Hansen 1989; Liu et al 1991; Oh et al 2002). According to our results, ethanol did not influence paracetamol metabolism in skin slices in-vitro. Although paracetamol can be metabolized in the skin in-vitro, no change in the rate of drug disappearance was noted with ethanol (Figure 5), indicating that alcohol functions as a penetration enhancer for paracetamol by physical mechanisms such as fluidizing the stratum corneum phospholipid layers. Nevertheless, this does not rule out a possible mechanism by which alcohol treatment reduces the skin temperature, resulting in a reduction of enzymatic activity and local paracetamol degradation in-vivo.

All the inactive ingredients utilized in the present study are widely used in pharmaceutical formulations and cosmetics. Glyceryl oleate and isopropyl myristate are generally regarded as non-irritant and non-toxic excipients. PEG-40 hydrogenated castor oil is a non-ionic surfactant used in a variety of oral, topical and parenteral pharmaceutical formulations, and it is essentially non-toxic and non-irritant. PEG-40 stearate is generally used as an emulsifier in oil-in-water type creams and lotions, and has also been used in intravenous injections and oral preparations.

Conclusion

We have shown that although paracetamol possesses poor skin permeability, it can be delivered transdermally in rats by appropriate formulation of safe, non-toxic and inactive chemicals. The use of both PEG-40 stearate and ethanol was found to be essential for the percutaneous absorption of paracetamol. The obtained plasma drug concentrations after patch application were comparable with those obtained after oral administration of a high drug dose but with a delayed profile.

References

Alberti, I., Kalia, Y. N., Naik, A., Bonny, J. -D., Guy, R. H. (2001) Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. Int. J. Pharm. 219: 11–19

Anderson, R. J., Kudlacek, P. E., Clemens, D. L. (1998) Sulfation of minoxidil by multiple human cytosolic sulfotransferases. Chem. Biol. Interact. 109: 53–67

Baker, C. A., Uno, H., Johnson, G. A. (1994) Minoxidil sulfation in the hair follicle. Skin Pharmacol. 7: 335–339

Berner, B., John, V. A. (1994) Pharmacokinetic characterization of transdermal delivery systems. Clin. Pharmacokinet. 26: 121–134

Berner, B., Liu, P. (1995) Alcohols. In: Smith, E. W., Maibach, H. I. (eds) Percutaneous penetration enhancers. CRC Press, Boca Raton, FL, pp. 45–60

Brunner, L. J., Bai, S. (1999) Simple and rapid assay for acetaminophen and conjugated metabolites in low-volume serum samples. J. Chromatogr. B: Biomed. Sci. Appl. 732: 323–329

Buhl, A. E., Waldon, D. J., Baker, C. A., Johnson, G. A. (1990) Minoxidil sulfate is the active metabolite that stimulates hair follicles. J. Invest. Dermatol. 95: 553–557

Campanero, M. A., Calahorra, B., Garcia-Quetglas, E., Lopez-Ocariz, A., Honorato, J. (1999) Rapid liquid chromatographic assay for determination of acetaminophen in plasma after propacetamol administration: application to pharmacokinetics studies. J. Pharm. Biomed. Anal. 20: 327–334

Chien, Y. W. (1987) Advances in transdermal systemic medications. In: Chien, Y. W (ed) Transdermal controlled systemic medications. Marcel Dekker, Inc., New York, pp. 1–22

Choonara, I. (1994) Percutaneous drug absorption and administration. Arch. Dis. Child. 71: F73–F74

DeNoble, L. J., Knutson, K., Kurihara-Bergstrome, T. (1987) Enhanced skin permeability by ethanol: mechanistic studies of human stratum corneum by DSC and FTIR. Pharm. Res. 4: 59S

Epstein, E. H., Lanston, A. W., Leung, J. (1988) Sulfation of the epidermis. Ann. N. Y. Acad. Sci. 548: 97–101

Evans, N., Rutter, N. (1989) Transdermal drug delivery to the newborn infants. In: Hadgraft, J., Guy, R. H. (eds) Transdermal drug delivery. Marcel Dekker, Inc., New York, pp. 155–176

Ghanem, A. H., Mahmoud, H., Higuchi, W. I., Rohr, U. D., Borsadia, S., Liu, P., Fox, J. L., Good, W. R. (1987) The effects of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin. II. A new quantitative approach. J. Control. Release 6: 75–83

Harpin, V. A., Rutter, N. (1983) Barrier properties of the newborn's skin. J. Pediatr. 102: 419–425

Hatanaka, T., Shimoyama, M., Sugibayashi, K., Morimoto, Y. (1993) Effect of vehicle on skin permeability of drugs: polyethylene glycol 400-water and ethanol-water binary solvents. J. Control. Release 23: 247–260

Insel, A. P. (1996) Analgesic, antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In: Hardman, J. G., Limbird, L. E. (eds) The Pharmacological basis of therapeutics, 9th edn. McGraw-Hill, New York, pp. 617–657

Kai, T., Mark, V. H. W., Potts, R. O., Guy, R. H. (1990) Mechanism of percutaneous penetration enhancement: effect of n-alkanols on the permeability barrier of hairless mouse skin. J. Control. Release 12: 103–112

Kuroki, T., Ikuta, T., Kashiwagi, M., Kawabe, S., Ohba, M., Huh, N., Mizuno, K., Ohno, S., Yamada, E., Chida, K. (2000) Cholesterol sulfate, an activator of protein kinase C mediating squamous cell differentiation: a review. Mutat. Res. 462: 189–195

Liu, P., Higuchi, W. I., Song, W. Q., Kurihara-Bergstrome, T., Good, W. R. (1991) Quantitative evaluation of ethanol effects on diffusion and metabolism of β-estradiol in hairless mouse skin. Pharm. Res. 8: 865–872

Miner, D. J., Kissinger, P. T. (1979) Evidence for the involvement of N-acetyl-p-quinoneimine in acetaminophen metabolism. Biochem. Pharmacol. 28: 3285–3290

Oh, S. Y., Fujii, M., Takeda, Y., Yoda, K., Utoguchi, N., Matsumoto, M., Watanabe, Y. (2002) The effect of ethanol on the simultaneous transport and metabolism of methyl p-hydroxybenzoate in excised skin of Yucatan micropig. Int. J. Pharm. 236: 35–42

Schachtel, B. (1992) Analgesic agents. In: Yaffe, S. J., Aranda, J. V. (eds) Pediatric pharmacology, 2nd edn. WB Saunders Co., Philadelphia, pp. 406–412

Shimada, M., Kamiyama, Y., Sato, A., Honma, W., Nagata, K., Yamazoe, Y. (2002) A hydroxysteroid sulfotransferase, St2b2, is a skin cholesterol sulfotransferase in mice. J. Biochem. 131: 167–169

Sintov, A., Gorodischer, R. (2000) Transdermal drug delivery systems. Israeli Patent Application 137,559

Walters, K. A. (1989) Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft, J., Guy, R. H. (eds) Transdermal drug delivery. Marcel Dekker, Inc., New York, pp. 197–246

Weibel, H., Hansen, J. (1989) Penetration of the fragrance compounds, cinnamaldehyde and cinnamyl alcohol, through human skin in vitro. Contact Dermatitis 20: 167–172